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Breast Cancer RESEARCH

SHORT COMMUNICATION

Systems pathology

Dana Faratian*

Introduction

Histopathology is a data-rich discipline. A standard histopathology report describing the macroscopic, microscopic, and basic molecular features of a breast cancer resection specimen contains tens of individual data items, including the histological type, size, grade, completeness of excision, node status, presence or absence of pre-invasive disease, oestrogen receptor (ER) and human epidermal growth factor receptor (HER)2 status, and sometimes a comment on response to pre-operative systemic therapy, to name a few. When this information is combined with clinical parameters such as age and co-morbidities, clinicians can, with remarkable accuracy, determine what the likely outcome is for an individual patient, and tailor treatment accordingly. However, in spite of thousands of articles documenting and claiming refinement of the morphological characterisation of breast cancers using single marker prognostic or predictive tissue biomarkers, only ER and HER2 are routinely used in clinical practice as predictive biomarkers of response to endocrine therapy and trastuzumab, respectively [1]. Markers of proliferation, such as measurement of the Ki67 antigen, may offer additional information but have yet to gain wide acceptance [2]. When performed at its best, basic histopathological examination of breast cancer remains the gold standard in determining patient outcome in breast cancer. Given the relative lack of success of new molecular clinical tests and the expansion of targeted therapies available to breast cancer patients, it seems timely to ask ourselves why tissue biomarkers fail to make a clinical impact, and to explore alternative strategies for biomarker discovery and individualised therapy.

From candidate pathology to systems pathology

The most common type of study demonstrating the effectiveness of a biomarker for prognosis or prediction of response to therapy in breast cancer is based on the candidate approach ('candidate pathology'). Sometimes, although by no means always, a candidate or group of candidate molecular targets are selected on the basis of a biological hypothesis that the molecule will in some way influence the biology of breast cancer, that is, by promoting apoptosis or reducing cellular proliferation. These hypotheses are sometimes informed by supporting studies *in vitro* or *in vivo*, but often the candidates represent the 'favourite' molecules of an investigator or laboratory. The past few years in particular have seen an explosion in the number of studies taking this approach, facilitated by the ready application of immunohistochemistry to tissue microarrays, which allow the simultaneous analysis of hundreds of tissue samples on a single glass slide [3]. Developing biomarkers based on solid biological reasoning has clearly been successful in a handful of cases - ER and HER2 most notably, and in ovarian cancer the exploitation synthetic lethality by poly(ADP-ribose) polymerase (PARP) inhibition in *BRCA* mutant tumours illustrates elegant rational predictive biology [4]. However, in the majority of cases these studies fail to make a long-term impact and are consigned to the literature archives without ever making it as far as independent validation, let alone clinical trials or the clinic.

The second most common type of study takes an unbiased approach to biomarker discovery using high-throughput methodologies, such as gene expression microarrays, to find statistical associations to define the biological characteristics (or differences) between cancers or to find statistical associations in the expression of genes, or groups of genes, and clinical outcome. This 'systematic pathology' approach has resulted in a deeper understanding of the heterogeneity of breast cancer [5], which has driven tailoring of therapy and new clinical trials for breast cancer subgroups, such as platinum-based therapy in triple-negative tumours, which are enriched for basal-like cancers [6]. This strategy has also led to the development of successful clinical tests, such as the OncotypeDX platform, which predicts long-term risk of recurrence in ER+, node-negative breast cancer, and which can help guide the decision on which patients to give chemotherapy to in the setting of early breast cancer [7].

However, in spite of the successes outlined above, the candidate and systematic pathology approaches also have their limitations. For example, HER2 has a relatively high

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negative predictive value but low positive predictive value; that is, it is good at selecting patients who will not respond to trastuzumab, but poor at selecting those who will [8,9]. This is because single target biomarkers are only one species in the complex signalling networks in which they participate [10]. This is exemplified by the signalling networks downstream of the HER2 receptor, particularly the phosphoinositol 3-kinase (PI3K) pathway, which when aberrantly activated (either through loss of PTEN or mutation of PIK3CA, which are frequent events in breast cancer and occur independently of HER2 amplification) contribute to trastuzumab resistance and insensitivity to other HER2-targeted therapies, such as pertuzumab [11]. Therefore, at the very least, effective predictive tests probably need to be multivariate and multiplexed in order to capture network complexity on an individual tumour basis.

Secondly, independent validation of biomarkers in appropriately powered clinical cohorts is often lacking, in spite of excellent recommendations and guidelines for robust validation of tissue biomarkers [12,13]. The availability of material for validation is not always forthcoming, or the fact is that the necessary trial to test a particular hypothesis for a particular therapy simply does not exist. Prospective testing in the clinical trial setting can be costly (particularly for high-throughput approaches), time-consuming (5 to 10 years to validate results for the desired clinical outcomes), and tests are likely to become redundant quickly in the face of rapid evolution of targeted therapeutics. Finally, even if a biomarker is identified as being able to stratify a patient population for prognosis or therapy at a statistically significant level, the magnitude of effect is not always sufficiently large to warrant change to an individual patient's treatment. For truly personalised therapy, either the effect has to be large or there needs to be an alternative or complementary therapy if the individual belongs to a particular subgroup.

Finally, the biomarker assay itself must be robust, but is not always so. Antibodies must be, above all else, specific, and validated using appropriate controls, which must be demonstrated. Immunohistochemistry, while the mainstay of pathological assessment of tissue biomarkers, is at best only semi-quantitative and subject to inter- and intra-observer variation, and measurement of biomarkers must follow the lead of validated quantitative gene expression assays. This may take the form of quantitative fluorescence analysis systems, such as the AQUA system [14], which has recently been recommended as a promising strategy for ER measurement by the National Comprehensive Cancer Network (NCCN) Task Force report [15] due to superior sensitivity, reproducibility, and quantification. Furthermore, biomarkers that show a large degree of tissue variability, due to either biological or artefactual heterogeneity, are unlikely to be reproducible.

So what can be done to complement the candidate and systematic pathology approaches, in order to overcome some of their limitations and streamline biomarker discovery? Recently, the nascent field of systems biology has entered the scientific lexicon as a promising strategy to understand complex biological systems [10,16,17]. Cancer is the archetypal complex system; each tumour is hardwired by different underlying genomic aberrations, cellular signalling represents a massively complex set of non-linear networks connected by feed-forward and feedback loops, each cell and tumour is spatially heterogeneous with respect to intracellular compartments (that is, nucleus, cytoplasm, intracellular organelles) and the extracellular environment (stroma, blood vessels, immediate microenvironment, oxygen tension) and the entire tissue is influenced by the host (that is, endocrine and immune systems, circulating factors, and metabolomics) [18]. Differences may also exist when the tumour spreads from the primary to distant sites [19]. Although systems biology defies accurate definition, for the purposes of this commentary, a systems biology approach includes a degree of mathematical modelling of biological complexity in order to describe changes in biology over time. Systems pathology is the application of these methods to human disease, and in particular breast cancer.

Systems pathology in breast cancer

In spite of the successful application of systems biology to other areas of clinical medicine, such as predicting the side effects of the drug ranolazine on the heart [20,21], systems biology has failed to gain widespread acceptance within the clinical academic community. This is perhaps surprising, since other abstractions of biology, which help to make complexity of disease more understandable, are accepted and commonplace. For instance, cancer cell culture systems help us to understand basic biological process and test biological hypotheses, even though they are not an absolute representation of cancer (epithelium, stroma blood vessels, and so on) itself. An additional problem is that there are relatively few 'success stories' of the useful application of systems biology to cancer medicine. So where might systems pathology be useful in breast cancer?

As stated above, although signalling in cancer is often represented by simple linear pathways, the reality is that signalling is complex and non-linear, with multiple levels of crosstalk and feedforward and feedback loops, which results in robust networks insensitive to perturbation, that is, therapy. Therefore, while cancer might be difficult to model, the need to do so is even greater since many new therapies are designed to target such signalling pathways. Failure to do so can be costly; for example, loss

of feedback inhibition in tumours treated with mammalian target of rapamycin (mTOR) inhibitors results in induction of AKT signalling, and may be responsible for the disappointing efficacy of mTOR antagonists in the clinic [22]. Negative feedback signalling mechanisms are likely to contribute to the poor efficacy of agents when studied in phase II and III cancer trials and to the high rate of attrition of drugs (approximately 30% due to efficacy), which is both time consuming and expensive [23]. Empirical testing of every possible agent or combination of agents in the preclinical or clinical setting becomes prohibitively expensive and impractical.

Process-based models (that is, those based on our understanding of the network biology, versus data-driven models) afford a mechanistic representation of the underlying cell dynamics and may be parameterised directly by experimental data. These models are formulated in terms of ordinary differential equations that describe the kinetics of the concentrations of molecular species within the network over time.

We recently employed a process-driven approach in order to study resistance factors to receptor tyrosine kinase (RTK) inhibitors such as trastuzumab and pertuzumab [24]. Since the reported resistance mechanisms to trastuzumab seem to relate to aberrant mitogen-activated protein kinase (MAPK)/PI3K signalling (*PIK3CA* mutations and inactivation of the tumor suppressor gene *PTEN* [25,26]), we reasoned that a systems analysis of these pathways, which are the best studied process-driven models to date, would be a useful application of systems biology to a clinical problem in oncology. A new model of MAPK/PI3K was developed to describe HER2-inhibitor antibody/receptor binding, HER2/HER3 dimerisation and inhibition, AKT/MAPK crosstalk, and the kinetic and regulatory properties of PTEN, and was based on modelling studies of the HER signalling network [27-30]. The inclusion of the tumour suppressor protein PTEN was deemed particularly important since it is a key negative regulator of the PI3K signalling pathway. We demonstrated that resistance to RTK inhibitors was governed by the PTEN:activated PI3K ratio (integrated resistance factor γ), and that PTEN, appropriately measured in the clinical setting, could stratify patients for HER2 inhibitor or combinatorial therapy, particularly an RTK inhibitor and PI3K inhibitor in cancers with low γ. This is one of few 'success stories' of how a systems biology approach can generate hypotheses that can be tested experimentally in preclinical models and that can then be applied to clinical evaluation. Such approaches might help increase the level of evidence that a particular biomarker might be useful within a prospective clinical trial.

Further examples of applied systems biology are required so that it might gain credibility and be accepted within the clinical community. In breast cancer, this need not be limited to cellular signalling. For instance, in spite of very accurate biochemical characterisation of the effect of cytochrome P450 enzyme polymorphisms and how they affect the rates of metabolism of tamoxifen [31], there is no formal mathematical description of these enzymatic processes. A unified model of tamoxifen metabolism to take into account individual genetic variability (measured in each patient before commencement of therapy) and the effect of dose and interacting agents (for example, known interactors such as selective serotonin reuptake inhibitors) could help tailor tamoxifen therapy on an individual pharmacogenomic basis. Models of host factors could ultimately be integrated with models of tumour biology (that is oestrogen signalling pathways), refining the individualised therapy approach to take into account drug pharmacokinetics and pharmacodynamics.

Conclusions - what is needed to make systems pathology reality?

The above discussion highlights some opportunities for using new mathematical methodologies for biomarker identification, drug target validation, and perhaps individualised pharmacogenomic approaches for breast cancer patients. Although a comprehensive description is outside the scope of this commentary, other mathematical techniques offer opportunities for integrating clinical and pathological data, such as Bayesian networks and fuzzy logic [10]. In tandem, because systems biology is a quantitative science, data generation in pathology needs to be refined and improved in order to generate high-quality data for systems pathology, which may come in the form of quantitative protein assays such as reverse phase protein arrays, quantitative immunofluorescence, mass spectrometry, or *in vivo* imaging. Clinical models in which multiple measurements of changing disease can be made are essential, such as extended neoadjuvant protocols with multiple biopsies or implantable devices (a 'lab-on-a-chip'). Finally, systems pathology is not mutually exclusive to other approaches used to discover or refine biomarker development, such as high-throughput approaches or empirical testing. Instead, systems pathology should be regarded as an opportunity to add value to data generation and generate new hypotheses about breast cancer biology, and increase the burden of evidence for the use of biomarkers in the clinic.

Abbreviations

ER, oestrogen receptor; HER, human epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositol 3-kinase; RTK, receptor tyrosine kinase.

Competing interests

The author declares that they have no competing interests.

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